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### Mapping of the SDHA Locus to Bovine Chromosome 20

T. S. Sonstegard

USDA-ARS, [tads@lpsi.barc.usda.gov](mailto:tads@lpsi.barc.usda.gov)

S. M. Kappes

USDA-ARS

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# Mapping of the *SDHA* locus to bovine chromosome 20

T S Sonstegard<sup>1</sup> and S M Kappes<sup>2</sup>

<sup>1</sup> ARS-USDA Gene Evaluation and Mapping Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA;

<sup>2</sup> ARS-USDA Roman L. Hruska Meat Animal Research Center, Clay Center, NE 68933, USA

*Correspondence:* T S Sonstegard (e-mail: [tads@lpsi.barc.usda.gov](mailto:tads@lpsi.barc.usda.gov)).

*Source/description:* The bovine *SDHA* cDNA (succinate dehydrogenase flavoprotein subunit A) has been cloned and sequenced<sup>1</sup>. From the published sequence (GenBank accession number [M60879](#)), primers [SDHA467](#) and 770 were designed to amplify across a potential splice site to find polymorphisms from an intron. The predicted product size from amplification of cDNA was 304 bp. Amplification of genomic DNA resulted in a 1202-bp product (GenBank accession number [AF139922](#)), which was sequenced to confirm proper amplification of *SDHA* alleles, intron size (898 bp) and splice donor site location (position 169 of [AF139922](#)). Exon and intron designations could not be determined, since all available *SDHA* sequences in GenBank are derived from cDNA. For polymorphism detection, a second primer pair ( [SDHA613](#) and 657) was used to amplify a 943-bp fragment that spanned the intron.

*Primer sequences:*

SDHA467: 5'-GGA GCT GGA GAA TTA CGG C-3'

SDHA770: 5'-GTG TTC CTG GCC CTG ATG-3'

SDHA613: 5'-TGC TGC ACA CGT TGT ATG G-3'

SDHA657: 5'-AGC TGG TGT CAT AGC GCA G-3'

**PCR and PCR-RFLP conditions:** PCR amplifications were performed on a PTC-200 thermocycler (MJ Research, Watertown, MA) in a 12- $\mu$ l reaction containing 20 ng of genomic DNA, 50 m m KCl, 1.5 m m MgCl<sub>2</sub>, 10 m m Tris-HCl (pH 9.0), 30  $\mu$ m each dNTP, 0.4  $\mu$ m of each primer, and 0.35 units of *Taq* DNA polymerase (Promega, Madison, WI). The profile for thermal cycling was, for 35 cycles: denaturation 94 °C, 15 s; annealing 62 °C (SDHA467 and 770) or 58 °C (SDHA613 and 657), 30 s; elongation 72 °C, 45 s. After amplification with SDHA613 and 657, 10  $\mu$ l of reaction mix containing NEBuffer 4 + BSA (final concentration 1 $\times$ ), and 1 U *Nla*III (New England BioLabs, Beverly, MA) was added to each sample before incubation at 37 °C for 1 h. Digested products were electrophoresed on a 3% agarose (1 $\times$  TBE) gel. Monomorphic product sizes were 192, 27, 383, and 97 bp; and the polymorphic sizes were either 20 (not visible) and 224 or 244 bp.

**Polymorphism:** Sequence analysis of the *SDHA* 1202 bp products derived from 12 parental animals of the USDA MARC reference population<sup>2</sup> revealed single nucleotide polymorphisms (SNPs) at sense strand position 863 (GGCCC A/G TGTGC) and position 978 (TCCCT C/G TCCCC). Both SNPs were detected only in animals of *Bos taurus* $\times$ *Bos indicus* descent.

**Linkage analysis and chromosomal location:** Genotypes from the reference population (76 informative meioses) were generated by PCR-RFLP detection of the A/G-862 SNP in the 943 bp *SDHA* product. Linkage analysis revealed that the *SDHA* locus maps 1.3 c m distal to *BMS521* (twopoint rec. freq. = 0.01, LOD 17.91). This result extends coverage of the linkage group 1.3 c m closer to the telomeric end of bovine chromosome 20 (BTA20). The human orthologue of *SDHA* is localized to HSA5p15, therefore the placement of bovine *SDHA* extends the syntenic conservation between BTA20 and HSA5q13.3-p14.3-5p15.

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